DRUG DELIVERY—VAGINAL ROUTE

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INTRODUCTION

Vaginal dosage forms have been developed and used clinically for many years in local therapy and the systemic delivery of systemically effective drugs. Pharmaceutical dosage forms available for intravaginal delivery consist primarily of those used to treat a specific gynecological condition. A small survey conducted to evaluate the preferences of females with regards to the use of intravaginal medications showed a positive market outlook for this mode of delivery (1). Products available include vaginal contraceptives, antifungals, antimicrobials, cleansers, deodorants, and lubricants. These products are formulated as tablets, capsules, creams, suppositories, foams, films, solutions, ointments, and gels. Currently, numerous prescription and over-the-counter (OTC) medications intended only for local activity in the vagina are available.

Recently, the vagina's absorption capacity has been recognized, which suggests that the vagina could provide a potential route for systemic drug delivery with a direct entry into the blood stream (Fig. 1) with the possibility of bypassing the hepatic-gastrointestinal (GI) metabolism. Several pharmacologically active compounds, that are metabolized extensively when taken orally, such as progesterone and estrogen, have been delivered intravaginally for achieving their systemic activity. Use of the vaginal route as a novel site for drug delivery has recently received greater attention, particularly with the new focus on the therapeutic agents that are subject to an extensive hepatic "first-pass" elimination, such as therapeutic proteins and peptides.

This article describes the physiology of the human vagina, its characteristics of absorption, and its permeability. The reader will also be familiarized with current research trends in vaginal delivery and absorption of drugs.

THE HUMAN VAGINA

General Anatomy

The vagina is a canal extending from the vulva to the cervix (Fig. 1). Extensive investigations on its morphology and anatomy have been compiled (2). Physiologically, the vagina serves a few functions, acting primarily as a conduit for the passage of seminal fluid, an excretory duct for menstrual discharge, and as the lower part of the birth canal (3). The anterior portion of the vagina in an adult averages 6–7 cm in length, while the posterior wall is approximately 7.5–8.5 cm.

The vagina is characterized by an exceptional elasticity, having the greatest resiliency at parturition. Along the length of the vagina, a layer of relatively thick connective tissue is located between the anterior vaginal wall and the urinary tract as well as between the posterior vaginal wall and the intestinal canal. The vaginal wall itself consists of three layers: The epithelial layer, the muscular coat, and the tunica adventitia. The epithelial layer is made up of an epithelial lamina and a lamina propria. It is a noncornified, stratified squamous epithelium that is subject to changes with aging. The epithelium atrophies from birth to puberty, at which time hormonal activity increases the thickness and resistance of this layer. In the subepithelial layer, there rests a network of elastic fibers around the lamina propria and collagenous fibers around the tunica adventitia, creating a connection to the muscular coat. Changes in the cytology of the vaginal epithelium occur with the cyclical stages in women. The epithelium is thickest in the proliferative stage, peaking at ovulation, and then diminishing with the secretory phase. The muscular coat of the vagina is composed of smooth muscle and elastic fibers. A spiral arrangement of these fibers provides support to withstand stretching without rupturing the vagina. The tunica adventitia is formed of loose connective tissue that is attached to the muscular coat. Fluctuations in

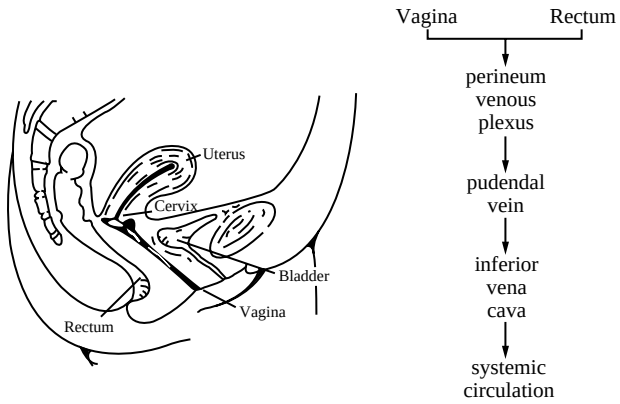


Fig. 1 Lateral view of the female pelvis showing the absorption route to the systemic circulation.

the volume of the vaginal lumen occur due to alterations in the tension of this layer. The vagina is encompassed by a vascular supply of arteries, veins, and lymph capillaries, as well as sensory and autonomous nerves.

Cellular structure

Histological studies of vaginal biopsies from healthy volunteers during follicular and luteal phases, postmenopause, and following ovariectomy have been conducted to characterize the ultrastructure of the vaginal mucosa by electron microscope (4). The epithelium of the vaginal mucosa is found to have five different layers of cells: The basal, parabasal, intermediate, transitional, and superficial layers (Fig. 2). The cellular types that make up these various layers renew continuously as they are stimulated by hormonal action and intracellular communication. The basal cells are typically columnar or squamous in shape with microvilli present on the surface of the cell membrane. Parabasal cells are similar to the basal cells in size and structure, but have a greater formation of surface microvilli and interdigitations. Their polygonal shapes are formed by adapting to spaces left free from neighboring cells. The cells of the intermediate layer possess microvilli and are of the largest cell type. The transitional cells that follow show noticeable signs of involution and surface characteristics of diminishing and thinning microvilli and intracellular junctions (desmosomes). Superficial cells, as indicated by their nomenclature, are the cells of the outermost layer during the follicular phase of the cycle.

The vaginal epithelium contains a network of intercellular channels that continuously undergo development, reaching a maximum during the ovulatory and

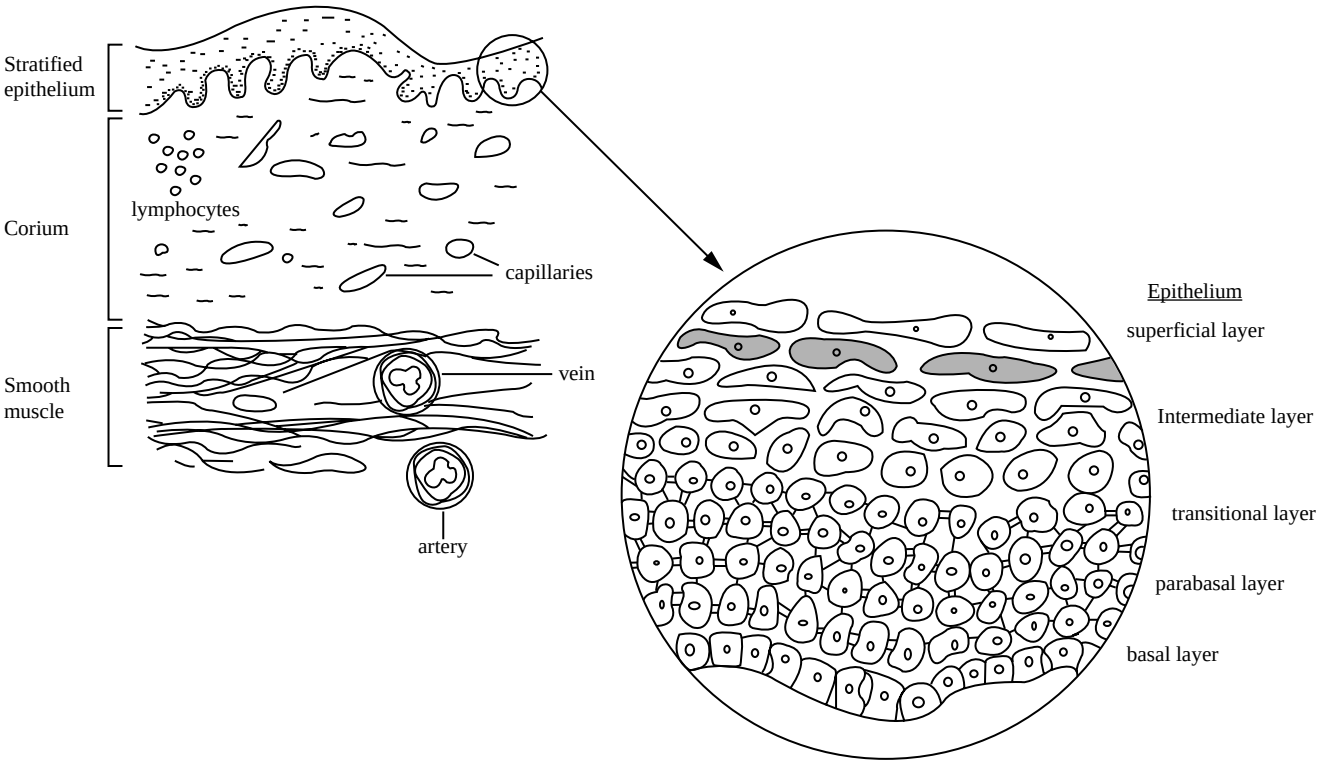


Fig. 2 Cross-sectional view of the vaginal wall with magnification of the stratified epithelial layers. (Based on Ref. 6.)

luteal phases. The channels present in the transitional and superficial layers do not change with the cycle as do those in the basal, parabasal, and intermediate layers. These channels provide a supply of nutrients and transport metabolites to and from the layers of the epithelium. Since the vagina is absent of secretory glands, lubrication is provided via these channels. Interestingly, different substances have been shown to be rapidly absorbed through the vaginal mucosa and will be discussed later. Intercellular junctions, including desmosomes and tight junctions, have also been identified. Desmosomes are most prominent in the intermediate layer and progressively become less toward the superficial layer, which may play a role in the desquamation of vaginal cells.

Since the vaginal epithelium is affected by ovarian hormones, cyclical variations can occur (Fig. 3). Although less intense than the uterine modifications, changes include proliferation, differentiation, and desquamation. During the follicular phase, the time period between the end of menstruation and the day of ovulation, mitosis increasingly occurs in the cells of the basal and parabasal layers, creating an increase in the number of layers and the thickness of the epithelium. The desquamating layers increase until ovulation, after which the layers diminish and are sloughed away through the vaginal lumen. During the luteal phase, the period after ovulation, the transitional cells become superficial due to the absence of the normal superficial layer.

It has been found that the basal cells replicate continuously to provide a self-cleaning mechanism to the epithelial layer. Autoradiographic studies of cell proliferation were performed on normal human cervix and vagina (5). The turnover time, an indication of the time required for the replacement of the cell population, was determined from these studies. Results showed the basal layer to be relatively inactive with a turnover rate of 33 days, while active proliferation occurred in the parabasal layers with a turnover rate of 3 days. The intermediate and superficial layers were found to be inactive differentiating compartments.

Fluids and enzymes

Despite the paucity of glands, the vaginal epithelium is usually kept moist by a surface film. This film, known as vaginal fluid, consists of cervical mucus and exfoliated cells from the vagina itself. Transudation from the blood vessels through the intercellular channels to the lumen can also contribute to the chemical composition (4). The fluid can contain carbohydrates, amino acids, aliphatic acids, protein, and immunoglobulins (Igs) (6). Nonserum proteins in human vaginal secretions have recently been

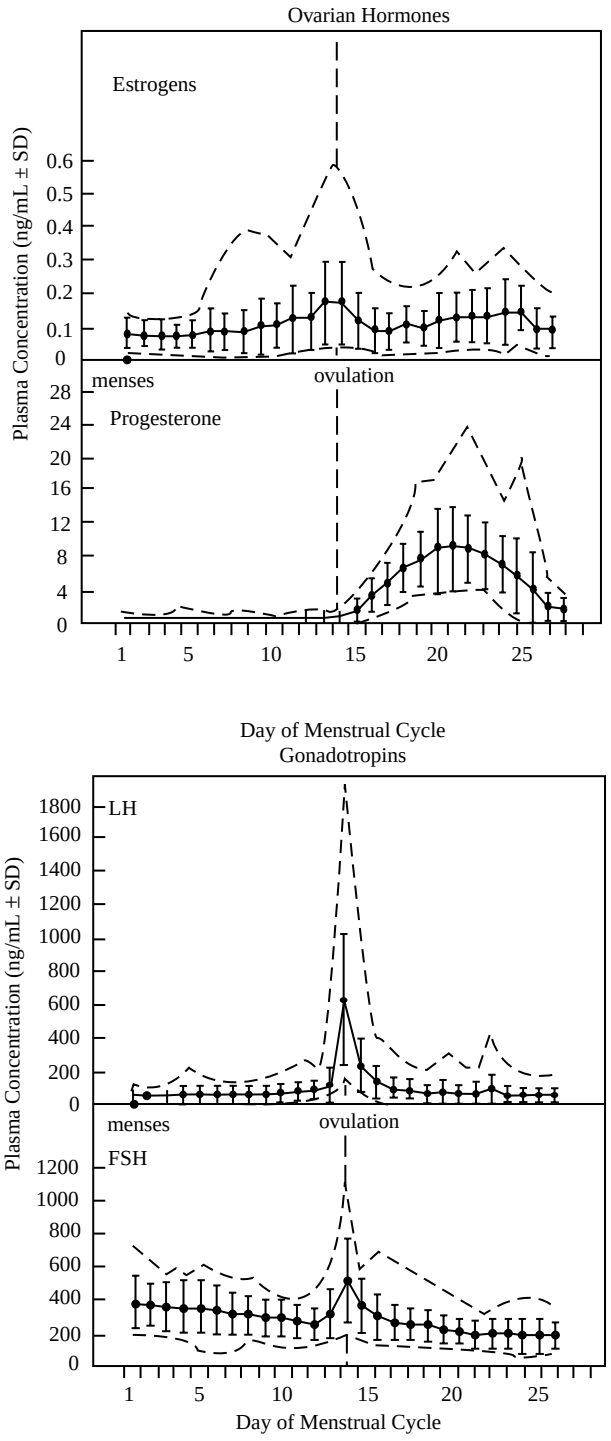


Fig. 3 Profile of the gonadotorpin and ovarian hormones during a normal menstrual cycle. The number of cell layers in the vaginal epithelium rises from 22 layers at approximately day 10 of the cycle to 45 layers at ovulation, and drops to 33 layers around day 20. (Based on Refs. 87 and 164.)

localized and analyzed (7, 8). Typically, the vaginal fluid in mature, healthy women has a pH in the range 4–5 (9). This acidic environment is produced by the presence of lactobacilli, which convert carbohydrates to lactic acid. The cervical mucus, a principal component of the vaginal fluid, is produced by glandular units within the cervical canal and has a pH in the range of 6.5–9. The cervical mucus changes in composition and physical characteristics with the menstrual cycle, facilitating sperm migration during ovulation. At the time of ovulation, the vaginal fluid increases in volume. This is due to the augmented amount of cervical secretions. The mucus produced at ovulation has increased spinnbarkeit (fibrosity), fering (crystallization of the mucus when dried on a slide), pH, and mucin content (10). Additionally, a decrease in the viscosity, cellularity, and albumin concentration is noted.

The variety of enzymes found in the vagina is an important concern for the development of vaginal delivery systems, particularly with proteases and their effect on protein and peptide candidates (11). The outer cell layers of the vagina contain varying amounts of β-Glucuronidase, acid phosphatase, α-Naphthylesterase, diphosphopyridine nucleotide-diaphorase (DPND), phosphoamidase, and succinic dehydrogenase (9). Enzymatic activity has been shown in the basal cell layers as well, and these layers contains β-Glucuronidase, succinic dehydrogenase, DPND, acid phosphatase, and α-Naphthylesterase.

In addition to enzymes, the vaginal lumen is a nonsterile area inhabited by a variety of microorganisms, mainly *Lactobacillus, Bateroides*, and *Staphylococcus epidermidis*, as well as potentially pathogenic aerobes (12). The existence of these microbes and their metabolites may also have a detrimental effect on the intravaginal stability of a vaginal drug delivery device.

Physiology and Dynamics

The unstimulated vagina anatomically consists of a luminal space that exists potentially rather than actually. However, in response to sexual excitement, some tension-induced anatomic variations occur. These anatomic variations may have effects on the long-term intravaginal delivery systems. These variations are reflected in the existence of four phases in a sexual response cycle as outlined by Masters and Johnson (13).

The first sign of physiological response (excitement phase) to stimulation is the production of a vaginal lubricating fluid. This appears on the vaginal mucosal surface within 10–30 sec after an effective stimulation. As sexual tension progresses, individual droplets of transudation-like mucoid material appear scattered throughout the rugal folds of the vaginal lumen, coalescing to form a smooth coating over the entire vaginal mucosa surface. This transudative mucoid material results from the activation of a massive localized vasocongestive reaction and marked dilation of the venous plexus that encircles the entire vaginal lumen. This sweating phenomenon provides complete lubrication of the vagina. The inner two-thirds of the vaginal lumen lengthen and distend. As sexual tension mounts toward the plateau phase, the vaginal wall in this area expands involuntarily and then partially relaxes in an irregular, tensionless manner. The demand to expand gradually overcomes the tendency to relax. In addition to the expansive effect in the vaginal fornices, the cervix and corpus pull slowly backward and upward into the false pelvis position. This cervical elevation creates a "tenting effect" at the transcervical depth in the midvaginal plane. This phenomenon always occurs in a normal anteriorly positioned uterus (Fig. 4). The vagina of either nulliparous or multiparous women, regardless of prior degree of vaginal expansion of lengthening, increases substantially in length and transcervial width with sexual stimulation.

With attainment of the plateau phase level of sexual tension, a marked localized vasocongestive reaction develops in the outer one-third of the vaginal lumen. The

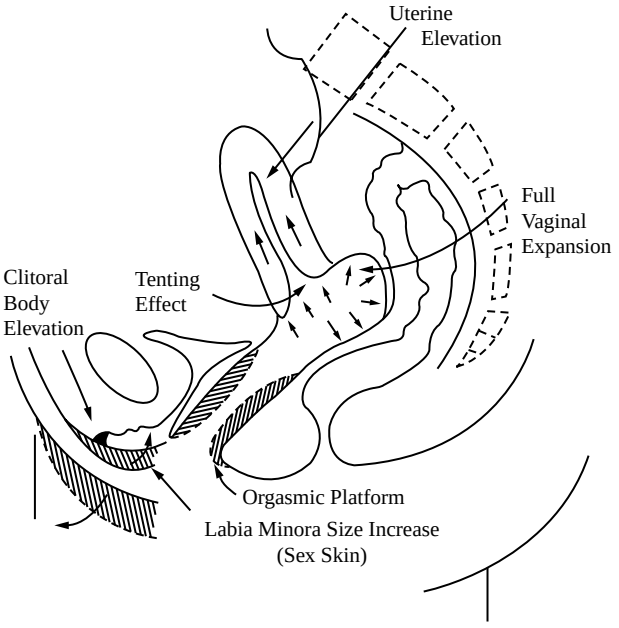


Fig. 4 Lateral view of the female pelvis showing the stimulated vagina. (Based on Ref. 13.)

entire area becomes grossly distended with venous blood, and its central lumen is reduced by at least a third as compared to the distention previously established in the excitement phase. The increase in the width and depth of the vaginal lumen is minimal. The production rate of vaginal lubricating fluid also gradually slows, particularly if this level of sexual tension has been experienced for an extended period of time.

During the orgasmic phase, the basic response of the inner vaginal lumen is essentially expansive rather than constrictive in character. On the other hand, the bulbar vasoconstriction at the orgasmic platform in the outer one-third of the vaginal lumen contracts strongly in a regularly recurring pattern. The intercontractile intervals lengthen in duration, and the intensity of the contractions progressively diminshes.

Along with the onset of the resolution phase, retrogressive changes develop first in the outer one-third of the vaginal lumen. The localized vasocongestion is dispersed rapidly, leading to an increase in the diameter of the central lumen of the outer one-third of the vagina. The previously expanded inner two-thirds of the vaginal lumen also gradually shrinks back to the original collapsed, unstimulated state. This shrinking process is an irregular, zonal-type relaxation of the lateral and posterior walls. The anterior wall and the cervix of the anteriorly positioned uterus descend rapidly toward the vaginal floor, leading to a quick resolution of the tenting effect created earlier during the excitement phase.

Menopause

The natural aging process results in significant changes in the vagina, including a decrease in vaginal size, loss of elasticity, loss of vascularity, and a thinning of the mucosa (14). The cytology of the vagina is variable and many aspects are addressed by Steger and Havez (14). The epithelium becomes markedly thinner and is often invaded with leukocytes. Areas can be completely denuded of an epithelial covering, exposing the subepithelial connective tissue. On the surface of the vagina, the numbers of exfoliating cells and microridges are greatly reduced. Creating the loss of elasticity, collagen replaces many of the elastic fibers in the lamina propria. Glycogen is very low or completely absent, contributing to the change in vaginal microbiology and pH. Vaginal secretions become scant and watery, and the pH increases from 4.5–5.5 to 7.0–7.4. Resistance to bacterial and fungal infections is reduced due to the lower population of acidophilic organisms (15). The enzymes present in the vagina also increase with the onset of menopause, including β-Glucuronidase, acid phosphatase, and nonspecific esterases (14).

Unlike other tissues, the vagina is greatly affected by steroid replacement therapy. Estrogen replacement therapy is often used to treat menopausal symptoms. The postmenopausal state of the vaginal epithelium, with its thinner epithelium and increased permeability, is an important consideration in drug delivery. The minimization of epithelial fluctuation will result in less fluctuation in absorption, affecting both systemic and local drug deliveries.

VAGINAL ABSORPTION

Much has been written in the literature concerning vaginal absorption. The first experimental studies using animals dates back to 1918. At that time, the histological characteristic of the vaginal wall was known to exist in three simple layers: the connective tissue, muscular, and the mucosa, collectively resembling the skin without the stratum corneum. Originally, the vagina was regarded as an organ impermeable to exogenous agents. Reports began to surface that indicated vaginal absorption of foreign materials as the cause of toxicity and even death in several cases. Using dogs and cats, vaginal absorptions of large varieties of compounds, including alkaloids, inorganic salts, esters, and antiseptics, were demonstrated (16). Later work showed the vaginal absorption of compounds such as hydrocyanic acid, pilocarpine, atropine, and insulin in dogs and cats (17). Using rabbits, cats, and dogs, the vaginal absorption of quinine bisulfate and oxyquinoline sulfate was set forth, intending to emphasize the care and consideration required of physicians with regards to the local applications of medication to the vaginal mucosa (18). A unidirectional transmission of agents was proposed to occur from the vagina to the blood, with no transmission in the reverse direction (19, 20).

An extensive review of the literature documented the absorption of carbohydrates, fats, and proteins (21, 21). Glucose was absorbed and rapidly oxidized. One of the first proteins demonstrated to be absorbed vaginally was peanut protein (23). The vaginal permeation of spermatozoa and bacterial antigens also has been shown (22). Bacterial antigens play an important role in triggering the local immunological mechanisms involved in protecting the area against infection. Other classes of compounds include steroids (e.g., estrogens, progesterone, and testosterone), prostaglandins, antimicrobials, nonoxynol-9, and methadone. The vaginal delivery of estrogen and

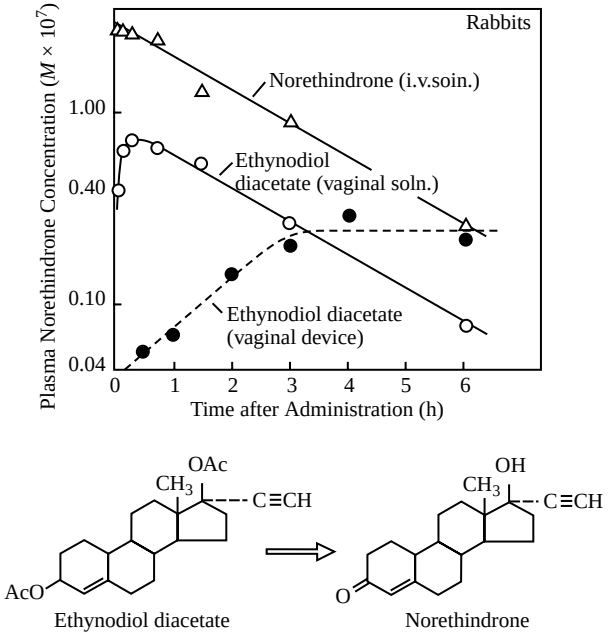


Fig. 5 Rabbit plasma concentration profiles of norethindrone following the intravenous administration of a single dose (solution). Also shown is the intravaginal absorption of ethynodiol diacetate from a solution dose and from a vaginal delivery device. (Based on Ref. 37.)

progesterone has been well documented over the years and used clinically in dosage forms, such as vaginal creams and suppositories.

Vaginal absorption of drugs is dependent upon such physicochemical properties as molecular weight, lipophilicity, ionization, molecular size, chemical nature, and local action, as well as the thickness of the vaginal wall as affected by the ovarian cycle or pregnancy (21). Other factors include changes in the vaginal epithelium and pH with menopause. Prior to absorption, drugs must be in solution. The fluid present in the vagina can help to dissolve drugs, but the cervical mucus secretion also can present a barrier and remove a drug from the site when abundant (11). Dosage forms can undergo different absorption due to the differing dissolution patterns in vaginal fluid. Products such as creams, inserts, and tablets remain for different periods of time in the vagina. A comparison of vaginal inserts versus creams showed that creams have a longer contact time in the vagina (24).

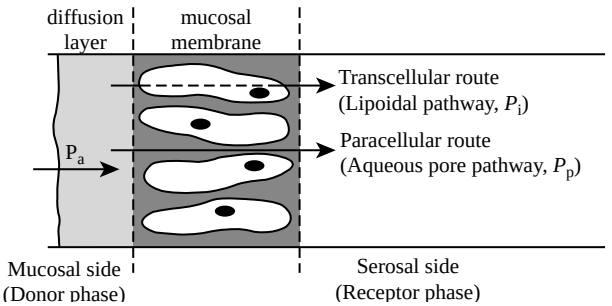


Fig. 6 Schematic of the vaginal membrane as a transport barrier. (Based on Ref. 25.)

Permeability

Transport across the vaginal membrane can occur by three primary pathways: the transcellular route, by which diffusion occurs through the cell due to a concentration gradient; the intercellular route, where diffusion occurs through spacing between cells; or by a vesicular or receptor-mediated transport (11, 25). Vaginal permeation studies have been conducted using the rabbit as an animal model (26, 27). The female rabbit does not exhibit an estrus cycle, so its vaginal tissues show constancy in the histological, biochemical, and physiological properties not ordinarily seen with most other mammals (28). The lack of a sexual cycle is, therefore, expected to produce a minimal variability in the permeability of the vaginal membrane, making the measurements of vaginal drug permeation more controllable and accessible (26, 27, 29).

The vaginal mucosa permeability of the doe rabbit has been examined by continuous perfusion of straight-chain alkanols and alkanoic acids (26, 27). Similar to the vaginal absorption of ethynodiol diacetate (Fig. 5), the vaginal uptake of both alkanols and alkanoic acids also follows a first-order rate process and is dependent on the drug concentration in the vaginal fluid. The results agree well with a physical model that has a hydrodynamic diffusion layer in series with the mucosal membrane, that consists of two parallel pathways: a lipoidal pathway and an aqueous "pore" pathway (Fig. 6). Immediately behind the mucosa (serosal side) a perfect sink is maintained by hemoperfusion.

The apparent permeability coefficient P_{app} for vaginal membrane permeation is defined by

$$P_{app} = \frac{1}{\frac{1}{P_{aq}} + \frac{1}{P_v}} \tag{1}$$

or

$$P_{app} = \frac{1}{\frac{1}{P_{aq}} + \frac{1}{P_p + P_l}} \tag{2}$$

since

$$P_v = P_p + P_l \tag{3}$$

where P_{aq}, P_v, P_p and P_l are the permeability coefficients of the aqueous diffusion layer, the vaginal membrane, the aqueous pore pathway, and the lipoidal pathway, respectively.

The vaginal permeation kinetics of a series of straight-chain alkanols was investigated (26). Using methanol as a reference permeant, a normalized permeability coefficient: P_{app} (*alc*/MeOH) was determined for each of the alkanols. The normalized permeability coefficient was observed to increase in value as the alkyl chain length of the alkanols increased (Table 1). The increased permeability can be attributed to the increase in the permeability coefficient for the lipoidal pathway P_l (Eq. 2). It is estimated that for straight-chain aliphatic alcohol the P_l value increases by 2.5 for the addition of each methylene (CH_2) group (26). On the other hand, the P_l value increases by 3.5 for the series of straight-chain alkanoic acids (27).

For the vaginal absorption of ionizable compounds, such as the homologous series of *n*-alkanoic acids, the apparent permeability coefficient P_{app} becomes pH-dependent and is defined by Eq. 4:

$$P_{app}(n, pH) = \frac{1}{P_{aq}(n)} + \frac{1}{\dfrac{[H]}{Ka + [H]} P_l^0 10^{n\pi} + P_p} \tag{4}$$

where n is the number of methylene (CH_2) groups in the alkyl chain; [H] is the concentration of protons; K^a is the dissociation constant of the acid; P_l is the permeability coefficient of the lipoidal pathway for the hypothetical acid with zero carbon atom ($n = 0$); and π is the methylene group incremental constant, that is, 3.5 for straight-chain alkanoic acids.

Table 1 Effect of alkyl chain length on the normalized permeability coefficients of straight-chain alkanols[a]

Alkanol	$CH_3(CH_2)_nOH$	P_{app}(Alkanol/MeOH)[b]
Methanol	$n = 0$	1.00
Propanol	$n = 2$	1.11
Butanol	$n = 3$	1.13
Pentanol	$n = 4$	1.20
Hexanol	$n = 5$	1.48
Heptanol	$n = 6$	1.91
Octanol	$n = 7$	2.15

[a]Based on data from Ref. 26.
[b]Normalized permeability coefficient = P_{app}(alcohol)/P_{app}(methanol). Mean value from three rabbits at pH 6.0 and 37°C.

Table 2 Effect of alkyl chain length and pH on the normalized permeability coefficients of straight-chain alkanoic acids

	P_{app} (Acid/MeOH)[a]		
Acids	**pH 3**	**pH 6**	**pH 8**
Acetic	1.22	0.73	0.25
Butyric	1.62	1.94	0.34
Hexanoic	1.89	2.06	0.81
Octanoic	1.74	2.49	1.24
Decanoic	—	—	1.26

[a]Normalized permeability coefficient = P_{app}(acid)/P_{app}(methanol). Mean value from three experiments involving different rabbits at pH 3, 6, and 8.
(Based on data from Ref. 30).

As illustrated earlier in the homologous series of *n*-alkanols, the normalized permeability coefficient of *n*-alkanoic acids also shows a dependence on alkyl chain length (Table 2). In addition, the straight-chain alkanoic acids demonstrate a pH-dependence in their normalized permeability coefficients (27). It should be pointed out that the rabbit's vaginal secretion has an effective pKa value of 6.3 ± 0.1. However, the rate of vaginal secretion is relatively small, which leads to a surface pH of around 2.0–2.1 (30). This acid surface pH affects the extent of dissociation of *n*-alkanoic acids and thus the magnitudes of P_l and P_{app} (Eq. 3).

The vaginal uptake of steroids has also been studied and follows a first-order rate process as well (31). The normalized permeability coefficient of steroids appears to be dependent upon steroidal structure (Table 3). The permeability coefficient across the vaginal membrane (P_v) also shows the same trend of structure dependence; the lipophilic steroids (progesterone and estrone) were better absorbed than the more polar steroids

Table 3 Vaginal permeation parameters of representative steroids

Steroids	P_{app}[a]	$P_v \times 10^4$ (cm/s)	$P_{aq} \times 10^4$ (cm/s)
Estrone	1.00	7.60	2.81
Progesterone	0.93	6.10	2.80
Testosterone	0.29	0.75	2.76
Hydrocortisone	0.23	0.58	2.79

[a]Normalized permeability coefficient = P_{app} (steroid)/P_{app} (methanol). (Based on data from Ref. 31.)

(hydrocor tisone and testosterone). However, the P_{aq} values, the permeability coefficient across the hydrodynamic diffusion layer, are very much the same among the four steroids (Table 3). For drugs with high P_v values, such as progesterone and estrone ($6.1–7.6 \times 10^{-4}$ cm/s), vaginal absorption is mainly controlled by their permeability across the hydrodynamic diffusion layer on the surface of the vaginal mucosa ($P_v > P_{aq}$). Conversely, for drugs with low P_v values, such as testosterone and hydrocortisone ($5.8–7.5 \times 10^{-5}$ cm/s), vaginal uptake is determined predominantly by their molecular permeation through the vaginal membrane ($P_v \gg P_{aq}$) (32). A linear relationship between the vaginal permeability and a series of progestins and their lipophilicities have been shown in vitro using rabbit epithelium (33).

The apparent permeability coefficient P_{app} is related to the first-order rate constant for the disappearance of drug from vaginal lumen (k_v) as follows:

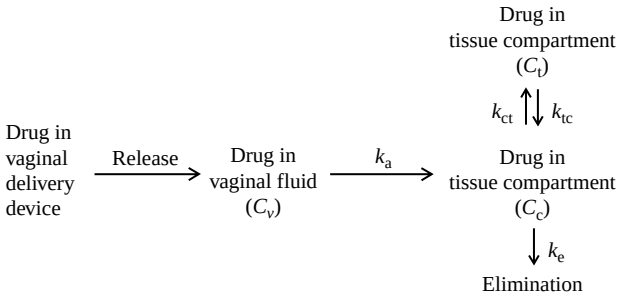
$$P_{app} = k_v \frac{V_v}{S_v} \tag{5}$$

where V_v is the volume of vaginal fluid and S_v is the geometric surface area of the vaginal lumen.

Additional permeability studies have been conducted in rabbits by the measurement of electrical conductance and flux measurements of a hydrophilic fluorescent probe 6-Carboxyfluorescein (34). Membrane permeation selectivity was indicated by KCl diffusion potential or charge-discriminating ability. The fluorescein probe is known to permeate via the paracellular route. The permeation of the 6-carboxyfluorescein was found to be in the order of intestine $\approx$ nasal $\geq$ bronchia $\geq$ tracheal $>$ vaginal $\geq$ rectal $>$ corneal $>$ buccal $>$ skin. The permeation selectivity was found to be negative; in other words, the K^+ ion was more permeable than the Cl. With all the tissue types evaluated, the permeation selectivity was found to be similar. Using low-molecular-weight polyvinyl alcohol, the molecular weight cutoff of the vaginal epithelium of rats was found to be greater than other assessable surfaces, such as the GI tract (35). Permeability trends have been evaluated using the spermicide, nonoxynol-9 (36). A linear correlation was observed between permeability and partitioning of nonoxynol-9 oligomers using lamb vaginal mucosa, suggesting the importance of the lipoidal pathway for this particular agent.

Pharmacokinetics

If the absorption, distribution, and elimination of a drug molecule after its release from a vaginal drug delivery device in the vaginal lumen follow the pharmacokinetic sequences,



then the instantaneous rate of change in drug concentration in the central compartment can be expressed by Eq. 6:

$$\frac{d(C_c)}{dt} = k_a C_v + k_{tc} C_t - (k_{ct} + k_e) C_c \tag{6}$$

where k_a, k_e, k_{cy}, and k_{tc} are the rate constants for absorption, elimination, and central compartment/tissue compartment exchange, respectively, and C_a, C_c, and C_t are the drug concentrations in the tissue fluid surrounding the vaginal device, in the central compartment and in the tissue compartment, respectively.

The vaginal absorption of drug following its release from vaginal drug delivery devices may alternatively be described by a simplified one-compartment open model with first-order drug absorption (Fig. 5) (37). Using this simplified model, Eq. 6 is reduced to Eq. 7:

$$\frac{d(C_c)}{dt} = k_a C_v - k_e C_B \tag{7}$$

where C_v and C_B are the drug concentrations in the vagina and in the body (including blood, tissues, and related compartments with fast drug exchange rates), respectively.

At steady state (Fig. 7), the change in the body concentration of the drug is relatively small, $d(C_B)/dt \approx 0$. For example, the body concentration of norethhindrone (C_B), the major metabolite of ethynodiol diacetate (Q), is then related to the amount of (Q) released at time t as shown by Eq. 8:

$$C_B = \frac{k_a \Sigma R_2 v}{2k_e} \left(\frac{Q}{t}\right)_v \tag{8}$$

where ΣR_v is the total diffusional resistance across the vaginal wall. Eq. 7 suggests that the norethindrone concentration (C_B) in the body of each test animal should be directly proportional to the amount of Q released from the vaginal device, $(Q/t)_v$, for a given duration of intravaginal residence. From the slope of the C_B versus $(Q/t)_v$ plots and the values of k_a and k_e from the vaginal absorption studies of the same drug from a solution, the

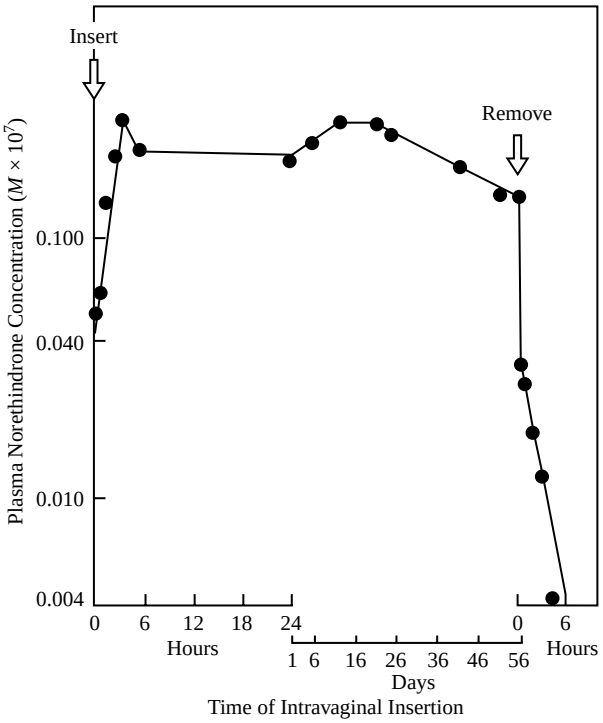


Fig. 7 Plasma profile of norethinedrone following the intravaginal insertion of ethynodiol diacetate-releasing vaginal devices in rabbits for 56 days and after device removal. (Based on Ref. 37.)

magnitude of R_v, the total diffusional resistance for the vaginal permeation of the drug, can be estimated.

Physiological Factors Affecting Drug Delivery through Vaginal Mucosa

Mucus: Compositions, characteristics, and roles

The vaginal mucosa, to which a bioadhesive drug delivery system is expected to come into contact when inserted intravaginally, consists of an epithelium having its surface coated with a layer of mucus. The mucus is a heterogeneous secretion and provides a protective and lubricating functions to the epithelium. In a normal woman, the mucus is produced by the cervix at the rate of 20–60 mg/day. During the midcycle, the rate increases to 700 mg/day (38) and the mucus becomes a less viscous and microstructurally more expanded in texture, which facilitates the penetration of sperm (39).

To understand the nature of mucus, it is necessary to understand the structure of glycoprotein, the principal biochemical component of the mucus subunit (40, 41). Glycoprotein, which has a molecular weight of 320–4500 Da, is bound to other subunits through disulfide bonds and probably interacts with other subunits through ionic bonds and entanglements, especially with sialic acids located at the terminal ends of the oligosaccharide chain (42). In addition to glycoproteins, the cervical mucus also contains a wide range of substances, including plasma proteins, enzymes, amino acids, cholesterol, lipids, and inorganic ions, with concentrations known to fluctuate during the cycle (43). It has been proposed that entanglement of the macromolecules with the specific lectin-like regions contributes to the properties of the cervical mucus (44, 45). The relationship between the intrinsic viscosity and the molecular weight of the whole mucins and their subunits and T-Domains suggests that they are flexible linear macromolecules behaving like a stiff random coil (46).

The hostility of the thickened cervical mucus to sperm penetration has been used as a means to achieve contraception, and low-dose oral contraceptives depend largely on this condition for their effectiveness in fertility control (47, 48). Sequential oral contraceptives do not affect mucus and like estrogen, may even increase sperm penetration.

The change of viscosity in the cervical mucus

Alterations in the biochemical properties of mucus are known to be responsible for changes in rheological behavior and receptivity of mucus to various exogenous compounds (49). The liquefaction can be regulated by mechanical disruption, systemic carrier dilution, or chemicals, such as mucolytic agents. The effect of the alterations in the physical and physiological properties of cervical mucus on the change in drug permeability through the cervical mucosa was studied (50). Since marked changes occur in the plasma membrane during epididymal maturation and capacitation, analysis of the nature of compounds at biochemical level was important to understand the changes in permeation rate in response to the viscosity of mucus (51). The increased ionic strength and consistency of the periovulatory mucus offers better permeability to exogenous compound, and increased charge favors a higher degree of hydration (52).

The viscosity of mucus is affected by binding between calcium and the mucus, which probably arises from an ionic interaction with the sialic acid in the mucin (44). These variations are indicated by a change in the pH, viscoelastic properties, water, and protein content of cervical mucus (53). Calcium is needed to establish an intercellular contact and the assembly of tight junction in the cervical epithelium (54). Changes in extracellular calcium affect the permeability of tight junctions and play a role in regulating the production of cervical mucus (55). Prostaglandin concentrations in the cervix affect the

viscosity of cervical mucus, which in turn affects the cervical softening (56). The effect of dithiothreitol (DTT) on the viscosity was also reported (57). Thus, the physical and physiological properties of cervical mucus may reflect alterations in the macromolecular composition or concentration of its components on exposure to exogenous substrates or hormone replacement therapy (58). As an example, administration of mestranol (or ethinyl estradiol), in combination with norethisterone or its acetate was known to strongly affect the biophysical properties of the cervical mucus and sperm migration (59).

The change in protein composition in cervical mucus

Human cervical mucus contains 1–3% proteins, in which the major soluble proteins are albumin and gamma globulin (60). The effects of various agents on the protein composition were studied (61). When a purified glycoprotein was treated with various proteolytic enzymes, which degraded (the purified) glycoprotein, the charge of cervical mucus increased to confer a rigidity by the mutual repulsion of negative charges and strengthen the coherence and consistency of the secretion (62). It appears that the menstrual cyclic changes in mucus viscoelasticity in an individual can be accounted for by the changes in mucin concentration (63). This appears to result from a decrease in the amount of proteins in both the follicular and the luteal phases, but an increase in the ovulatory phase (64). In addition, mucus compositional differences may occur among individuals, as indicated by the different correlations seen in the viscoelasticity and the mucin concentration (65).

The effect of the combination of nonoxynol-9 and EDTA was studied and showed that spermicidal activity was significantly enhanced (48). The observations may be due to a partial removal of calcium, on addition of chelating EDTA, which may have caused a decrease in the dephosphorylation of regulatory proteins mediated by calcium, and further caused a change in the total amount of protein and its composition in the cervical mucus. The nature of mucus components, especially proteins and enzymes, need to be studied further to fully elucidate the mechanism by which cervical mucus regulates the permeation rate of exogenous compounds. A change in the protein composition is expected to affect enzyme activity in the cervical mucus and further affect the stability and permeation rate of a drug delivered by a vaginal drug delivery device (66).

Cyclic variability

As mentioned previously, the rabbit appears to be an ideal animal model for studying vaginal mucosa permeation due to the absence of an estrus cycle. Unfortunately, the doe rabbit may not be a suitable animal model for studying long-term vaginal absorption because it lacks the typical cyclic variations observed in the human vaginal tract associated with the rhythmic pattern of hormones during a menstrual cycle. In the human female, the cyclic secretion of estrogenic hormones in the ovarian cycle induces variation in the histology, biochemistry, and physiology of vaginal tissues. It is, therefore, reasonable to expect that the vaginal mucosa undergoes a corresponding cyclic variation in its membrane permeability.

The macaque rhesus monkey has an ovarian cycle of approximately 28 days, as does the human female, and it also exhibits an estrus pattern very similar to the menstrual pattern of the human female. It is widely believed by researchers in the fertility field that rhesus monkeys and humans have comparable anatomy and physiology, as well as similar reproductive functions (67). Therefore, the female rhesus monkey is a superior animal model for studying the vaginal absorption of various drugs from a drug delivery system designed for use in human females.

The effect of the estrus cycle on the permeability of the vaginal mucosa has been demonstrated in the vaginal absorption of a small molecule, like methanol, which has a vaginal membrane-controlled permeation, and a larger molecule, such as *n*-octanol, with vaginal permeation controlled by the hydrodynamic diffusion layer (Fig. 8). Further studies that used intact and ovariectomized monkeys could not establish any systematic relationship between the menstrual cycle and vaginal membrane permeability (68). Conflicting observations were also reported in the vaginal absorption of penicillin in humans (69, 70) as well as in rats (71, 72).

The vaginal permeability of a cycling monkey during the period immediately following menstruation is lower than that of a noncyclic rabbit (Fig. 9). The difference in vaginal permeability between rhesus monkeys and rabbits is greater for hydrophilic molecules, such as the short-chain alkanols (e.g., methanol), whose vaginal permeability is controlled by vaginal membrane permeation. The difference lessens as the alkyl chain length of alkanols increases, since molecular lipophilicity increases at the expense of hydrophilicity. At ovulation, the monkey's vaginal permeability is several-fold lower than that of the noncyclic rabbit (31).

The cyclic variation in vaginal drug permeability observed in rhesus monkeys in association with the rhythmic pattern of the sexual cycle suggests that the vaginal absorption data generated in the rhesus monkeys may be more reflective of what will occur in humans. The rhesus monkey is, therefore, a good animal model for the research and development of intravaginal delivery devices.

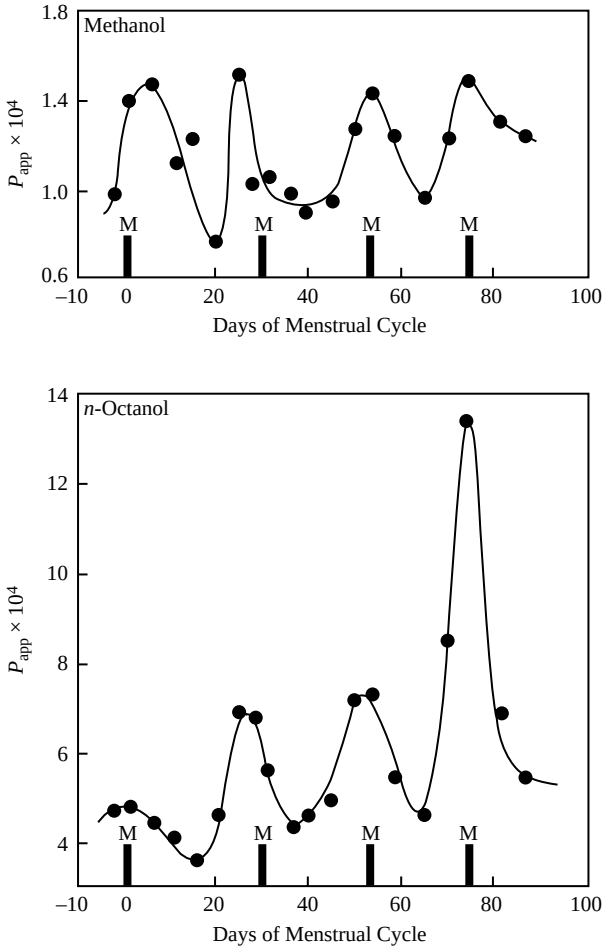


Fig. 8 Cyclic variation in the apparent permeability coefficient, P_{app}, of methanol and *n*-octanol in the female rhesus monkey in response to its estrus cycle. The bars indicate the time of observed menstruation. (Based on Ref. 31.)

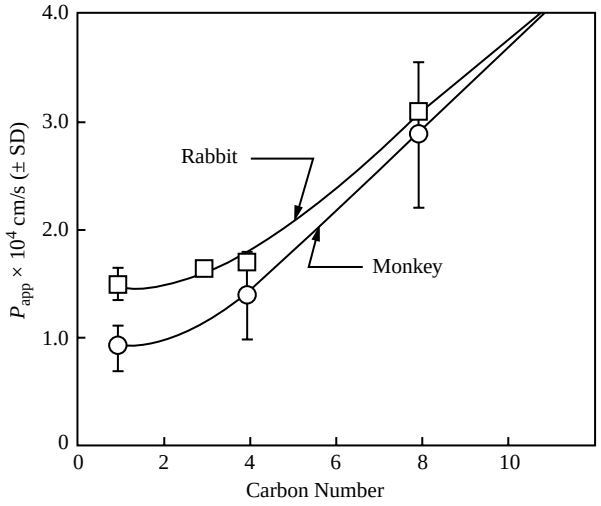


Fig. 9 Comparison of apparent permeability coefficients, P_{app}, for the vaginal absorption of straight-chain alkanols in noncyclic rabbits and cyclic rhesus monkeys. (Based on Refs. 16 and 165.)

TYPES OF VAGINAL DRUG DELIVERY SYSTEMS

Human Applications

Several publications have examined the many types of vaginal delivery systems already marketed or under development (73, 74). In the development of vaginal dosage forms, the following considerations should be addressed (73):

- Maintenance of an optimal pH for vaginal epithelium
- Ease of application
- Even distribution of drug
- Retention in the vagina
- Compatibility with coadministered medicines

In addition, offensive odors, staining, tissue irritation, or pain during sexual intercourse are undesirable. With regard to systemic delivery, one key advantage of intravaginal administration is avoidance of the presystemic elimination associated with oral dosage forms. The perineum venous plexus, which drains the vaginal tissue and rectum, flows into the pudenda vein and ultimately into the vena cava, which circumnavigates the liver on first–pass. This is in marked contrast to GI blood circulation that drains into the portal vein and passes directly through the liver before entering the systemic circulation. The vaginal route is preferable for drug entities associated with GI irritation and for the localized treatment of vaginal disorders that require minimal systemic absorption.

Creams, foams, and jellies

Many OTC vaginal products are available in these types of preparations. Common products include contraceptive creams, foams, gels, suppositories, sponges, and films, that contain an active spermicidal agent, like nonoxynol-9 or octoxynol. Vaginal preps are a recent FDA-approved OTC treatment for yeast infections (75). For treatment of menopausal symptoms, estrogen products are available as vaginal creams. A relatively new product for postmenopausal women, Replens®, is available as a vaginal bioadhesive moisturizer. It has been shown to be as safe and effective as estrogen vaginal creams in increasing vaginal moisture, fluid volume, elasticity, and returning the pH to the premenopausal state (76). Low pH lactate gels have been dispensed for the treatment of bacterial vaginosis. Application of these gels leads to a

disappearance of abnormal discharge and malodor, restores normal acidity, and facilitates recolonization with lactobacilli. Local treatment for vaginosis by the intravaginal delivery of antimicrobials may be preferred over an oral regimen, particularly during pregnancy (77).

When the efficacy of a vaginal ring, which delivers a continuous low dose of estradiol, was compared with a vaginal cream, both formulations were found to be equally effective and safe in the treatment of postmenopausal women with urogenital atrophy symptoms (53).

Vaginal rings

Vaginal rings provide a means of delivering a pharmacologically active agent to the systemic circulation at a controlled rate of release. The vaginal rings developed to date are primarily used for contraception and have been reviewed in the literature (74, 78). Compounds delivered include medroxyprogeterone acetate (MPA) (79–85), estradiol (53, 58, 86, 87), norgestrel (88, 89), levonorgestrel (90–94), combinations of progestins and estradiol (95), and combinations of progesterone and estrogens (96–101). Another area of interest is in the controlled delivery of prostaglandin for cervical ripening and induction of labor or pregnancy termination (102).

An advantage of intravaginal controlled drug administration over conventional oral administration is best illustrated in Fig. 10. After oral ingestion, MPA, reaches a peak plasma drug concentration rapidly within 2 h and declines over the next 22 h. On the other hand, intravaginal controlled delivery of MPA from a vaginal ring attains a steady plasma plateau within 4 h, which is maintained throughout the course of treatment until removal of the ring. The continuous "infusion" of drugs through the vaginal mucosa can prevent the possibility of hepatic GI first-pass metabolism and inefficient therapeutic activity resulting from the alternatively surging and ebbing plasma drug levels that occur with the intermittent use of oral dosage forms (31). Vaginal rings have been shown to be safe and effective for the delivery of estradiol and have been found to be more comfortable than a pessary (103). Local estradiol delivery via vaginal ring, vaginal cream, or suppositories, was reported to alleviate urogenital estrogen deficiency symptoms in postmenopausal women (53, 58). In both cases, patients showed a strong preference for the vaginal ring over other vaginal dosage forms. Danazol, that was administered intravaginally also via the vaginal ring was found to be very effective in the treatment of pelvic endometriosis (104). Danazol was absorbed through the vaginal mucosa and reached the deeply infiltrating endometriosis via diffusion.

Vaginal rings are made of biocompatible silicone elastomers that consist of a drug-free core ring and a drug-containing coat. Vaginal rings are inserted and positioned around the cervix. Those designed for contraception, are kept intravaginally for 21 days and removed for 7 days to allow menstrual flow. The vaginal ring was redesigned due to frequent bleeding irregularities. The new generation of sandwich-type vaginal rings contains a drug-dispersed silicone polymer matrix is coated by a nonmedicated silicone polymeric membrane. The design reduces the initial spike of drug release frequently observed in the first treatment cycle of vaginal rings for contraception. The effect of the overcoat on the release rate profile of d-norgestrel is demonstrated in Fig. 11, which shows that the addition of an overcoat minimizes or eliminates the burst release of drug and shifts the non-zero-order drug release profile to the constant zero-order release rate profile. The concept of intravaginal dual

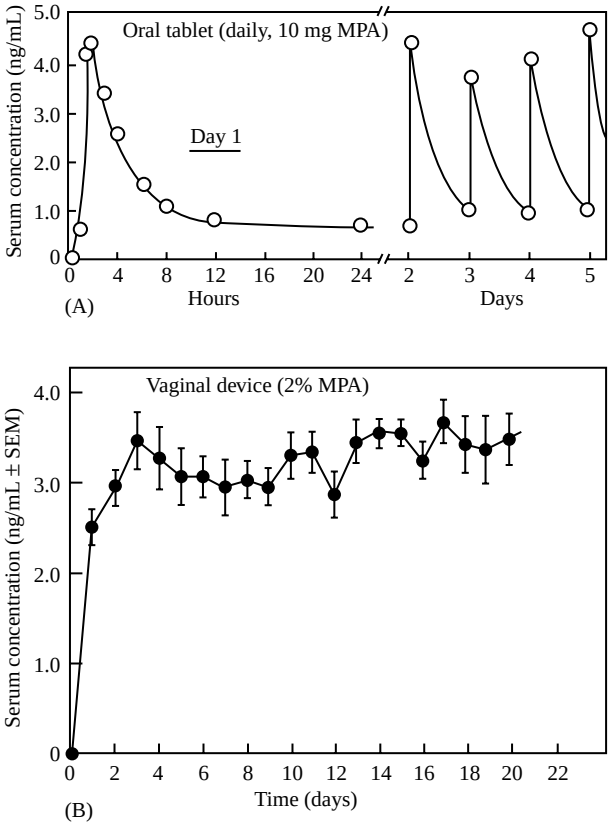


Fig. 10 A) Serum concentrations of medroxyprogesterone acetate after a daily oral administration of a medroxyprogesterone acetate tablet (10 mg) taken by a healthy woman before breakfast for five consecutive days. B) Daily serum concentrations of medroxyprogesterone acetate in women wearing medicated silicone vaginal rings (2% medroxyprogesterone acetate) for 20 days. (Based on Ref. 53.)

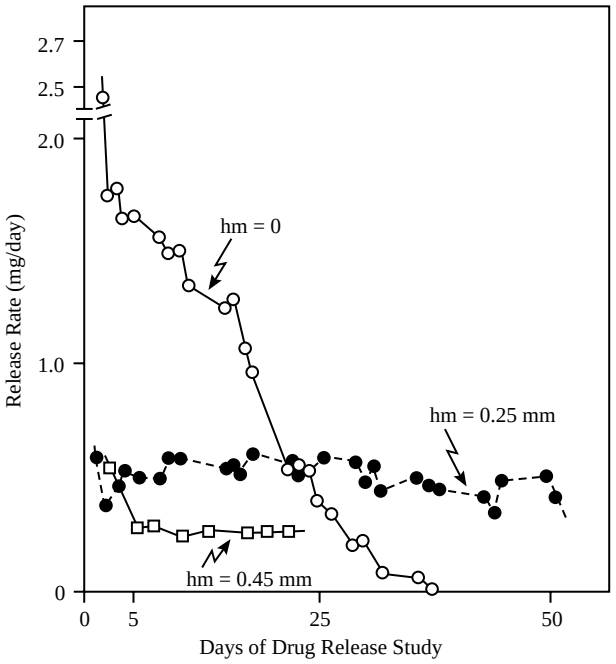


Fig. 11 Comparative in vitro release rate profile of levonorgestrel from a vaginal ring containing a homogeneous dispersion of drug in a silicone-based polymer matrix (open circles) and from one containing an inert overcoat covering the drug reservoir layer (closed circles and squares). The effect of overcoat thickness h_m on the release rate of levonorgestrel is also shown. (Based on Ref. 25.)

administration of progestin and estrogen in combination was recently extended to the development of a combined contraceptive vaginal ring. This new design (Fig. 12) is constructed from two drug reservoir compartments; the major compartment consists of a 3-Keto-desogestrel loaded core, and the other, minor compartment consists of a core loaded with a combination of 3-Keto-desogestrel and ethinylestradiol, a synthetic estrogen. These drug

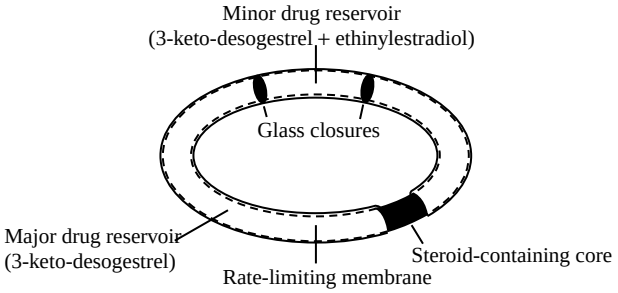


Fig. 12 Structural components of a contraceptive vaginal ring containing the combination of 3-keto-desogestrel and ethinylestradiol. (Based on Ref. 67.)

reservoir compartments are separated by two steroid-impermeable glass closures, as the partitions, and release the steroids at a fixed ratio through a rate limiting silicone membrane (98). Serum profiles of 3-Keto-desogestrel and ethinylestradiol from two prototype vaginal rings are shown in Fig. 13.

Veterinary Applications

Pessaries

Intravaginal drug delivery has been a useful tool in animal husbandry to control the estrus cycle of sheep and cattle. Synchronization of a herd estrus cycle eases the management strains on ranchers and farmers. Fluorogestone acetate, an effective ovulation inhibitor in the ewe, has been formulated into a vaginal pessary for sheep. The pessary is placed in the ewe for about 15 days. The pessary is removed and the sheep regain their estrus and ovulate within 2–4 days. This permits insemination of all sheep within a 2-day period and with a high success rate. Commercial delivery systems include Syncro–Mate vaginal pessary (S.D. Searle & Co.), Chronogest vaginal pessary (Intervet S.A.), and PRID vaginal insert for dairy cattle (Sanofi Animal Health Ltd.), which contains progesterone and estradiol benzoate. A MPA sponge has been used to evaluate the preovulatory gonadotropin surge in ewes (105).

Modified vaginal pessaries

The Syncro–Mate progestin-releasing vaginal pessary is fabricated by dispersing a progestin, such as fluorogestone acetate, in a pessary made of porous polyurethane sponge. The pessary can be readily inserted intravaginally and removed according to a predetermined schedule. The vaginal pessary has been redesigned with the aim of minimizing the loading dose, to overcome the matrix-type release and absorption profiles, and to improve systemic bioavailability. Work evaluating a cylindrical, drug-free polyurethane vaginal sponge coated by a laminate of fluorogestone-containing silicone matrix and a drug-free silicone coating membrane was conducted (106–108).

The system itself was modified and the release rates studied in vitro and in vivo. It was found that the drug-containing silicone layer needed to be in contact with the vaginal wall. A silicone coating did not result in adequate release rate. Also, as the surface area of the drug-containing silicone increased, the drug delivery rate increased. This effort has resulted in two new pessary designs (Fig. 14). Both types make use of the polyurethane sponge in the vaginal pessary as the mechanical support for vaginal insertion and retention, but

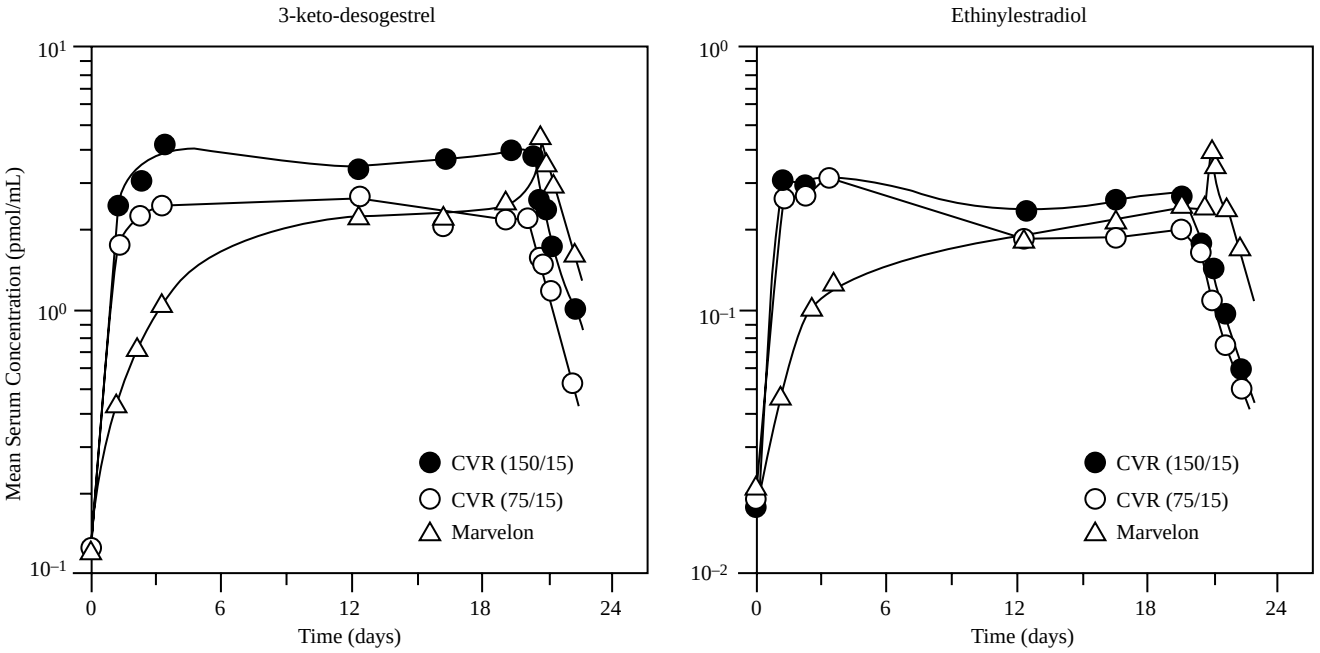


Fig. 13 Mean serum profiles of 3 keto-desogestrel and ethinylestradiol after a 21-day continuous intravaginal administration of two prototype combination contraceptive vaginal rings (CVR). The profiles from an oral combination tablet (Marvelon) is plotted for comparison. (Based on Ref. 67.)

the drug reservoir is relocated from the porous sponge matrix to a sheet-type rate-controlled silicone device that covers the circumferential surface of the sponge. The type I rate-controlled silicone device consists of a homogeneous dispersion of drug in a silicone polymer matrix. The type II drug-dispersing polymer matrix is sandwiched between two sheets of silicone polymer membrane to form a three layered laminate.

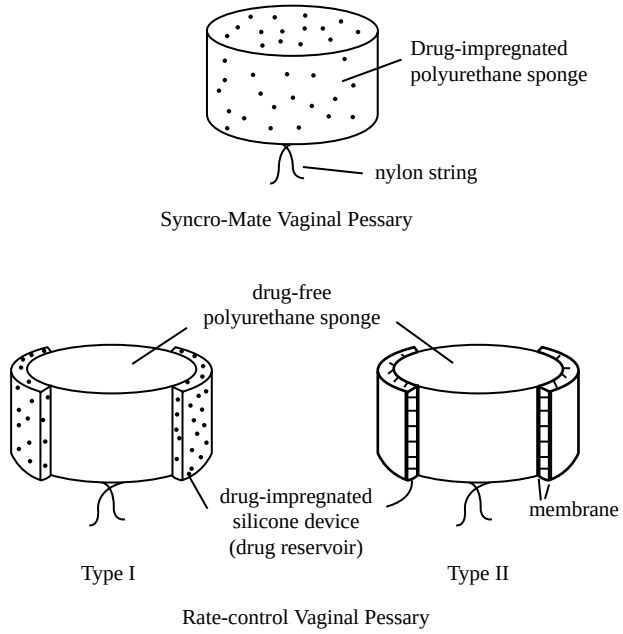


Fig. 14 Comparison of newer progestin-releasing vaginal pessaries (Type I and II) with an older (Syncro-Mate) pessary design. (Based on Ref. 25.)

Potential Developments in the Future: Bioadhesives

Novel intravaginal delivery systems include those that employ bioadhesive materials. The bioadhesive properties of compounds such as hydrogels may provide a controlled delivery system with a prolonged residence and intimate contact in the vagina. Many hydrophilic polymers and hydrogels have been used in vaginal products. These include starch, collagen, proteins, gelatin, and cellulose derivatives (hydroxypropyl methylcellulose, hydroxypropyl cellulose, and sodium carboxymethylcellulose) (77). Two synthetic hydrogels that have been reported in applications for vaginal drug delivery are poly(ethylene oxide) and poly(acrylic acid). Bioadhesion is thought to involve an initial interaction of the hydrogel with the mucosal surface, which requires a matching of the polarity between the tissue surface and the polymer surface (109), and subsequently, an interpenetration of the mucosal surface by the polymer chains of the hydrogel.

A review of vaginal bioadhesive formulations indicates that bioadhesive tablets have been used for localized treatment of diseases in the vaginal tissue (77, 109). For example, Bleomycin, an antitumor agent, was incorporated into a flat-faced disk fabricated from a combination of hydroxypropyl cellulose and poly(acrylic acid) (Carbopol 934) (110). The tablet was designed to release Bleomycin at a slow rate to minimize irritation to healthy mucosa. Another vaginal tablet is formulated from the combination of poly(acrylic acid) with hydroxypropyl methylcellulose and ethylcellulose (111). Other polymer combinations evaluated for potential bioadhesive vaginal delivery include poly(acrylic acid) and sodium carboxymethyl cellulose with Avicel PH102 (methylcellulose) as the diluent (112). Insulin has been formulated in a cross-linked poly(acrylic acid) gel and found to be adsorbed onto the vaginal surface of alloxan-induced diabetic rats and rabbits (113).

Emulsion-based formulations with bioadhesive properties also have been designed to deliver antifungal agents, although little has been reported in the literature (78). Bioadhesive microparticles used in nasal and oral delivery have the potential for further development of intravaginal delivery systems. The mucoadhesive benzyl ester of hyaluronic acid has been used in preparing microspheres for the intravaginal delivery of salmon calcitonin to rats (114). Replens®, which has been marketed as a bioadhesive moisturizer and which remains in the vagina for 2–3 days, consists of a bioadhesive cross-linked polycarbophil (115). Carbopol 934 polymer could be a good bioadhesive candidate for clinical application in the intravaginal delivery of spermicidal agents (44).

Liposomes, a novel drug delivery system, are widely applied in the topical treatment of diseases, including vaginal diseases. Their applications in contraceptive systems for the intravaginal administration of progesterone (116) and interferon-α (117) (or metronidazole) (118) for the genital papilloma virus infections were previously reported.

DRUG CANDIDATES FOR VAGINAL ADMINISTRATION

Antimicrobials

Antimicrobials, along with antifungals, have been important drug candidates for the treatment of vaginal diseases, such as bacterial vaginosis. Earlier researches on antimicrobials, such as penicillin and sulfanilamide, has been reviewed (22). Evaluations have been made on such drugs as metronidazole (119–121), clindamycin (122, 123), feticonazole (124), and clotrimazole (124, 125). Metronidazole has been prescribed for the treatment of amoebiasis, trichomoniasis, lambliasis, and anaerobic infection (121). It is administered vaginally as a gel, normally twice a day every 12 h for 7 days for the treatment of bacterial vaginosis. The gel results in lower serum concentrations with negligible adverse systemic effect (119). Metronidazole's low lipid solubility probably contributes to its poor vaginal absorption (120). A comparison of vaginal and oral drug delivery of metronidazole from a film-coated tablet showed that the maximum serum concentrations attained by vaginal delivery are only sufficient to kill the most susceptible anaerobes (121). One recent study confirmed that liposomes could be used as a novel delivery system for metronidazole in the treatment of vaginal infections (118).

Clindamycin also is indicated for the treatment of bacterial vaginosis Its efficacy as a 2% cream is similar to oral metronidazole treatment (122). Measurable amounts of clindamycin, corresponding to frequency of application, have been detected systemically at levels well below those of intravenous delivery (123). Gentamicin is an additional aminoglycoside that was evaluated for vaginal administration in ovariectomized rats (126). The results indicated the following order of absorption enhancers by their effectiveness: 1% palmitoylcarnitine $>$ 0.5% lysophosphatidylcholine $>$ 1% laureth-9 $>$ 10% citric acid. Severity of desquamation of the vaginal epithelium were scored as lysophosphatidylcholine $=$ laureth-9 $>$ palmitoylcarnitine $>$ citric acid.

The market for vaginal candidiasis treatments has been revamped in recent years due to the availability of OTC products. Recently, oral antifungal agents, i.e., ketoconazole, have been approved for treatment of vaginal candidiasis. Earlier research that compared vaginal feticonazole to oral clotrimazole (125) and vaginal clotrimazole to oral fluconazole (100) have found them equally effective and well tolerated as compared to the oral product used for comparison. An important consideration in the choice of oral vs.vaginal treatment is the contraindication of oral dosage forms during pregnancy. Vaginal treatment for recurrent vaginal conditions may be a good alternative in such a case (127).

Anticancer Agents

Intravaginal investigations into the local treatment of cervicovaginal cancers have made some progress over recent years. [^{3}H]-Fenticonazole has been evaluated in normal cervicovaginal mucosa, cervical carcinoma, and relapsing vulvovaginal candidiasis, and was found to be devoid of risk to patients (128). For patients with vaginal

rhabdomyosarcoma and residual vaginal disease following surgery and chemotherapy, the effects of high-dose irradiation from vaginal molds loaded with iridium[192] were examined (129). Individualized molds were made with rapid setting silastic foam and loaded with iridium wires. The patients treated remained well and disease-free for 7 years. The pharmacokinetics properties of ciclopirox olamine have been evaluated after vaginal application to rabbits and patients. The intravaginal absorption was found to be low while the penetration of drug was found in deep tissue. These results led to good local and systemic tolerability (130). Cisplatin suppositories have been tested preoperatively via vaginal administration (131). The Cisplatin penetration of the tumor surface needs further improvement in order to achieve effective local chemotherapy.

Contrary to cancer treatment, cervicovaginal cancers are linked to vaginal pessary use. Review of cancer cases found that tumors occurred at sites of ring insertion. Although chemical carcinogenesis cannot be ruled out, chronic local infection may be the main etiologic factor. The severity of cancer cases has declined in recent years due to the availability of more advanced surgical and radiation treatments with minimal complications (132).

Prostaglandins

For many years, prostaglandins, primarily prostaglandin E_2 (PGE_2), have been studied for cervical ripening and induction of labor as well as an abortifacient. Most of the prostaglandin delivery systems have been in the form of suppositories or pessaries (133–143). More recent work with pessaries and gels has been done due to the use of prostaglandins as an alternative to surgical abortions.

Early work with PGE_2 formulated a sustained-release pessary that used a swelled, cross-linked polymer hydrogel (143). Further work was conducted using a semicrystalline poly(ethylene oxide) and poly(urethane) hydrogel swollen with PGE_2 solution (144). The controlled release of PGE_2 and the cervical score showed a linear release, suggesting the advantage of control over cervical ripening (145). Pessaries provided greater control in labor induction (146), (more favorable than intracervical gels (147)), had high efficacy and low incidence of side effects (148), and showed a reduction in cesarean rates associated with artificial rupture caused by oxytocin infusion (149). Although PGE_2 has been shown to initiate active labor and reduce the need for oxytocin, it has been shown to cause uterine hyperstimulation or fetal heart rate abnormalities, which are reversed with removal of the pessary (150). Tablet forms of PGE_2 are shown to be more chemically stable and cost effective (151, 152). Additionally, the case of use may allow for shortening of postdate pregnancies in a safe and effective manner on an outpatient basis (153).

In addition, PGE_2 has been formulated as a gel. A comparison of tablets and a triacetin-base gel showed more favorable induction with the gel (154). The use of PGE_2 gel in women with prelabor spontaneous rupture of membranes, significantly improved the time of delivery without influencing the cesarean section rate or fetal-maternal infective morbidity (155). The intravaginally administered gel was more efficacious than that given intracervically (156). In regards to a regimen, either 12 h or 6-hourly, the majority of 12-hourly subjects achieved labor after a single dose, but the induction delivery interval was similar in both groups (157).

Due to its labor-inducing characteristics, prostaglandins also have been used as abortifacients. PGE_1, as a vaginal pessary, has been combined with the oral antiprogestin, mifepristone (RU486), and has been found to be a safe, efficient nonsurgical outpatient method of termination (158). The PGE_2 product, originally intended for the treatment of GI ulcers caused by nonsteroidal antiinflammatories, was shown to be a safe and low-cost method. Misoprostol alone has been used for the induction of labor with females with late fetal death (159). Attention of the medical profession and the news media has resulted in the use of misoprostol and methotrexate for early abortion. In this application, methotrexate, originally used as a chemotherapeutic agent in cancer and arthritis, is given intramuscularly followed by intravaginal misoprostol 3 days later. This is an effective abortion method for a gestation period of 56 days or less (160).

Spermicides

Spermicidal activity in the vagina is intended for fertility control, by eliminating the motility of sperm and ultimately killing them. Spermicides have become more popular with the rise in social awareness and prevention of sexually transmitted diseases. Many compounds have been evaluated for spermicidal activity. As mentioned, nonoxynol-9 and octoxynol are predominantly available in the United States. A few products that have been evaluated include alkyloxynol-741, which was tested in stump-tailed macaques for spermicidal activity, and gramicidine, which is used as a spermicidal agent in Russia but not in the United States. Alkyloxynol-741 was compared with nonoxynol-9 and chlorhexidine, using a dissolvable polyvinyl alcohol film, and found to be an inexpensive alternative in countries where nonoxynol-9 may not be readily available (161). The combination of nonoxynol-9 and EDTA in a gel formulation, developed from carbopol 934 polymer showed a significant

enhancement of efficacy in fertility control (44). A tablet form drug delivery system fabricated by incorporating nonoxynol-9 into polyvinylpyrrolidone, was reported to provide a short- and long-term release of nonoxynol-9 and produce an immediate and extended enhancement of the contraceptive properties (162). Protectaid, a contraceptive sponge marketed in Canada, contains sodium cholate, nonoxynol-9, and benzalkonium chloride. Sodium cholate itself has been shown to exhibit strong spermicidal and antiviral activity, and offers a new and modern protective method (2828 163). A serine protease from sperm, 44-acetamidophenyl-4-guanidinobenzoate, inhibits acrosin activity and has been found to be more potent and less irritating than nonoxynol-9, as well as providing protection against HIV (164).

Various studies found that the absorption of nonoxynol-9 through the vaginal membrane was very slow and suggested a dependence of molecular weight of the oligomeric components that make up the spermicide (165, 166). Permeation studies have found the hydrophilic–lipophilic balance (HLB) of the oligomers to play a role as well (36). In delivery gel made of calcium chloride cross-linked alginate containing 3% of nonoxynol-9, it was found that pH of delivery gel had a significant effect on spermicidal efficacy of nonoxynol-9 (167). This behavior could be attributed to nonoxynol-9 micelle formation.

Steroids

The vaginal delivery of steroids for urogenital symptoms has been shown to be more appropriate than oral and parenteral administration. Hormone replacement therapy is indicated for peri- and postmenopausal women suffering from vasomotor symptoms, vaginal dryness, and discomfort due to urogenital atrophy and other related symptoms of hormone deficiency. Steroids, progesterone and estrogen, have been used for many years to treat a variety of physiologic conditions, including hormonal replacement and contraception. As an alternative to oral estrogen replacement, vaginal estrogen cream is an effective treatment for vaginal atrophy (168). Use of estradiol cream has been effective for atrophic vaginitis (53) and also for endometrial proliferation and hyperplasia if coadministered with progestins (169). Systemic absorption of progestins could have the risk of endometrial hyperplasia.

Like estrogen, progesterone has been delivered via the vagina as creams, pessaries, and vaginal rings. Vaginal absorption and local redistribution of progesterone was observed in a study using young female pigs (170). Vaginal delivery has shown enhanced progesterone delivery to the uterus when compared with a standard intramuscular regimen (171). Micronized progesterone in a nonliquefying vaginal cream is promising (172, 173). For systemic delivery of progesterone to the genital organs, doctors have prescribed to their patients a suppository compound of progesterone and cocoa butter. A lactose-based progesterone tablet has been designed to deliver biologically effective amounts of progesterone for up to 48 h (77). These tablets form a milky suspension and stay resident in the vagina for a longer period of time, making them ideal for the treatment of menstrual irregularities, functional uterine bleeding, luteal phase defects, premenstrual tension, infertility, and osteoporosis. A recent study demonstrated that a "first uterine pass effect" occurred when progesterone was delivered intravaginally, thereby providing an explanation for the unexpectedly high uterine concentrations relative to the low serum concentration observed (174). Application of liposomes as the vaginal delivery system for progesterone to achieve contraceptive efficacy was successfully demonstrated (116). The combined use of progesterone delivered from an intravaginal-targeted drug delivery system and estradiol delivered from a transdermal therapeutic system were found to be very effective in producing artificial cycles (95).

Absorption and secretion characteristics of sodium prasterone sulfate have been evaluated in rats after vaginal administration. The absorption was significantly affected by the estrus cycle and the progression of the pregnancy (175).

Proteins and Peptides

Research on the intravaginal delivery of peptides and proteins has focused on insulin and gonadotropin-releasing hormones (GnRH) and their absorption into the systemic circulation. Insulin and thyroid-stimulating hormone have been shown to achieve some absorption from the vagina of rats and rabbits, but with a high dependency on the estrus cycle (113). Hydrophilic molecules, like insulin and GnRH, may be absorbed through intercellular channels, hence absorption would be greater when the epithelium is thinner (77, 176). As commonly observed with protein and peptide research, an enhancer is often needed to assist in absorption. The enhancers are limited in human testing; thus, most of the preliminary research must be done in animal models, such as the rat and rabbit (77). Vaginal administration of insulin was found to increase hypoglycemia in rats when using enhancers such as Na taurodihydrofusidate, polyoxyethylene-9-lauryl ether, lysophosphatidylglycerol, lysophosphatidyl choline, and palmitoylcarnitine chloride (177). Other therapeutic agents that have been investigated include leucine enkephalin (66), salmon calcitonin (115, 178), and

recombinant human relaxin (179, 180). Relaxin, structurally related to insulin, was formulated as a 3% methylcellulose gel for intravaginal delivery. In this form, it had limited permeability through nonpregnant rabbit and rhesus monkey vaginas.

Much work has been done using luteinizing hormone releasing hormones (LHRH) analogs, particularly leuprolide. Initial work with leuprolide found greater potency in rats via vaginal administration over rectal, nasal, and oral administration (181). Enhancement of absorption by organic acids (citric, succinic, tartaric, and glycocholic) increased bioavailability by 20%. The vaginal absorption from jellies was found to be a practical dosage form. Additional investigation of the organic acids used for enhancement was found to work by the acidifying and chelating ability (182). Citric acid also was shown to loosen the blood–vaginal epithelium barrier. The down regulation of the pituitary by chronic intravaginal treatment of leuprolide exhibited regression of hormone-dependent mammary tumors in rats (183–185). The vaginal route appears to be the preferred route according to a recent presystemic metabolism study of first-order LHRH degradation in rabbit homogenates (186). The degradation half-life of vaginal homogenates was 9–12 times longer than that of rectal homogenates and 3–4 times longer than that of nasal homogenates.

Vaccines, Antigens, and Gene Delivery

Factors that have a significant impact on immune responses are the route and method of vaccine or antibody delivery (187). Since the recognition of acquired immunodeficiency syndrome (AIDS) in the early 1980s, researchers have focused on the discovery of pharmacological agents for the treatment and ultimate cure of AIDS, a well as on preventing the spread of the virus. Mucosal infection via the vagina and rectum are reportedly two of the major pathways through which HIV and other sexually transmitting viruse are disseminated (188, 189). Mucosal immunity has been considered as the first line of immunological defense against these pathogens to prevent the systemic infection. The important factors to be considered in the development of vaccines are protection with a minimum number of administrations and a practicability of the approaches in inducing immunity at the mucosal surface (81, 190). Consequently, studies were conducted to elucidate the effects of variations in the routes of immunizations on the type and extent of mucosal response (191). Mucosal immunity in the female reproductive tract was reportedly influenced by immunoglobulins (Igs), cytokines, and reproductive hormones. The types of responses elicited following a DNA immunization were found to depend on both the identity of the antigen and the route of DNA administration. The epidermal delivery route, including vaginal mucosa, was found to be more efficient in terms of dosage requirements (192, 193). When the effects of exogenous hormones on reproductive tract immunity were evaluated in women on oral contraceptive pills (OCPs), the mean values of IgA in the cervical mucus of woman on OCPs were much greater than those in the naturally cycling women (194). The increased levels of IgA in the cervical mucus of women on OCPs may contribute to a lower incidence of sexually transmitted diseases. Using animal models, the transfection of mucosal tissues by gene–gun administered plasmids was demonstrated in vivo, and vaginal immunization of human growth hormone was noted to yield a higher titer of cervicovaginal antibodies than other routes of immunization (88).

DNA-based vaccines have been recently developed for immunization to overcome the deficiencies of antigen-based vaccines. The major sites of delivery for DNA-based immunization include the oral, nasal, rectal, and vaginal mucosas (195, 196). The ability of a hepatitis B surface antigen-encoding plasmid to induce responses in mice through the various routes of delivery was compared (197). It was reported that delivery through the vaginal route still produced a cytotoxic T lymphocyte activity, even though it failed to induce antibodies. A human simian virus 1 (HSV) vaccine was tested as an aqueous solution or gel intranasally, vaginally, and subcutaneously in guinea pigs (198). The gel system used was a controlled release carbopol gel. The animals were challenged 3–5 weeks later with only the subcutaneous response producing IgG and IgA. The nasal and vaginal routes showed that the vaccine could take up and elicit antibodies, thereby slightly reducing the severity of the disease, but showed no superiority to the subcutaneous route. In another study, rats were immunized with a synthetic peptide from a HIV envelope glycoprotein and shown to have greater IgG and IgA response with an enhancer, lysophosphatidyl glycerol (199, 200). The serum antibodies from subcutaneous and intravaginal delivery were able to recognize the glycoprotein (HIV 1 gp120), but no neutralizing activity against the virus was seen. An antigen delivery system of lysophosphatidylcholine and degradable starch microspheres demonstrated potential intravaginal delivery to sheep (199, 200). If the vagina is capable of mounting an immune response, antibodies in genital secretions may be able to reduce the transmission of HIV. Intravaginally administered tracers using (fluorescein isothiocyanate) (FITC) bovine albumin, FITC-horseradish peroxidase, and FITC-horse ferritin have shown the vagina and cervix to be major sites of protein uptake (201).

Lactobacilli from the female genital tract have been developed as a vehicle to deliver continued doses of foreign antigen to the vaginal mucosa surface with the aim of stimulating a local immune response (202). The lactobacillus fermentum was chosen for genetic manipulation and delivered intravaginally in guinea pigs and was maintained for 5 days. Despite the short period, the novel vaccination approach shows potential for stimulating mucosa immunization. Horse ferritin was combined with aluminum hydroxide, muramyl dipeptide, monophosphoryl lipid A, dimethyl dioctadecyl ammonium, and cholera toxin (203). The aluminum hydroxide combination was found to be the most effective. However, the doses of antigen used were larger than normal, and consequently the drug combination may be inefficient at more realistic dosages. Additional work with mouse ferritin has shown pelvic immunization at nonmucosal sites to be very effective in stimulating an IgA response in the female reproductive tract, more so than intravaginal delivery because of possible involvement of iliac lymph nodes not associated with the vagina (204).

As a potential means of treatment of gynecological conditions, *Candida albicans* vaccine was developed and an antibody response after intravaginal application of vaccine and effects on recurrent urinary tract infections were evaluated (205, 206). Serum antibody to some of non-*E. coli*, but no antibody to *E. coli* were expressed (207). The vaccine was well tolerated and invites further development. Another evaluated vaccines contained inactivated polio vaccine delivered by intravaginal, intrauterine, mesopharyngeal, and intramuscular routes. The predominant secrectory antibodies to polio viruse in the vagina were found to be IgA and IgG in the uterus. The genital tract is immunologically reactive and may play a role in protection against other infections such as gonnococcus and genital herpes (208). For veterinary use, dairy cattle research has directed attention to bovine herpes virus type 2, which causes ulcerative lesions in teats and udders. Vaccinations subcutaneously and intravaginally have been shown to be beneficial in reducing the severity of the infection and show potential for a vaccine for dairy cattle (209).

Anti-inflammatory Agents and Others

Bromocriptine has been used in the treatment of hyperprolactinaemic women (210). Drug in tablet form was administered intravaginally and found well absorbed from the vagina, with avoidance of side effects. For Benzydamine, a nonsteroidal anti-flammatory with local anesthetic and analgesic properties, mouthwash, dermal cream, and vaginal douche have been formulated and evaluated for local therapy (211). Another chemical entity that has been researched for intravaginal delivery is tranexamic acid, an antifibrinolytic drug usually given by mouth for the treatment of menorrhagia associated with intrauterine devices (IUD). The intravaginal delivery of tranexamic acid was found well tolerated with avoidance of GI side effects (212).

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